REASSESSMENT OF BIOWISH ACTIVATION PROCEDURE FOR DENITRIFICATION

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William Wah Yee

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COMMITTEE MEMBERSHIP

TITLE:	Reassessment of Biowish Activation Procedure for Denitrification
AUTHOR:	William Wah Yee
DATE SUBMITTED:	December 2013
COMMITTEE CHAIR:	Nirupam Pal, PhD Professor of Environmental Engineering
COMMITTEE MEMBER:	Amy Howes, PhD Associate Professor of Biology
COMMITTEE MEMBER:	Christopher Kitts, PhD Professor of Microbiology

ABSTRACT

Reassessment of Biowish Activation Procedure for Denitrification

William Wah Yee

BiOWiSH^{TM} – Aqua is a blend of preserved multi-bacteria culture with the capability of denitrification. If an anaerobic nitrate rich activation procedure is used instead of the standard aerobic activation procedure, the denitrification rate is increased by 28 percent under the conditions of 30°C, 1C:1N, 200mg/L of carbon, and 200mg/L nitrogen.

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Introduction

Denitrification is the microbial process in which nitrate is utilized as an electron accepter and converted into nitrogen gas. The process is facilitated by either heterotrophic or autotrophic bacteria. In a heterotrophic metabolism nitrate follows the reduction path of(Bedmar):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

During heterotrophic denitrification, exogenous organic carbon is used as an electron source. The organic carbon can be supplied as either methanol or glucose. With glucose as the electron donor and potassium nitrate as the electron accepter the overall denitrification process can be expressed by the following equation:

$$5C_6H_{12}O_6 + 24KNO_3 \rightarrow 24KHCO_3 + 6CO_2 + 12N_2 + 18H_2O$$

For unhindered denitrification, the dissolved oxygen concentration must be in the range of 0mg/L to 0.5 mg/L(Yoo H.). Furthermore, potassium bicarbonate is generated from the denitrification process allowing for auto regulation of the optimum pH (7.0-8.2).

BiOWiSH^{TM} – Aqua (Biowish) is a blend of preserved multi-bacteria culture developed for wastewater treatment. Biowish has been proven to contain bacteria capable of denitrification (Lee). As Biowish is freeze dried for shipping an activation process must occur before Biowish can be used to remove nitrates from an aqueous solution. The established activation procedure requires 10.0g of Biowish powder to be mixed with 2.0L of tap water and maintained at 30°C for 48 hours under well mixed conditions (Lee).

Nutrients in the form of nitrates released into natural water bodies can cause eutrophication, while nitrates released into drinking water sources can cause human health concerns such as methemoglobinemia more commonly referred to as blue baby syndrome. Biowish offers a method of denitrification which does not require the usage of expensive membranes or absorption media. Therefore Biowish can be a viable alternative for both drinking water and industrial waste water treatment.

If Biowish is activated in an anaerobic-nitrate environment of 400mg/L-N potassium nitrate, denitrifying bacteria will out compete the non-denitrifying bacteria in the Biowish culture; therefore increasing the denitrification rate after activation. The standard activation method was compared to the anaerobic-nitrate activation method and an anaerobic activation method.

Methods

Standard Activation

The standard activation procedure involves the mixing of 10.0g of Biowish with 2.0L of tap water with an incubation time of 48 hours at 30°C. The mixture was placed in a 2L Erlenmeyer flask for agitation in an orbital shaker. The orbital shaker provides both mixing and dissolved oxygen during the activation process.

Anaerobic-Nitrate Activation

The anaerobic-nitrate activation procedure involves the mixing of 10.0g of Biowish with 2.0L of tap water and 5.77g of potassium nitrate (800mg of nitrogen) with an incubation time of 96 hours at 30°C. The mixture was placed in a 2L media bottle and sealed in preparation for agitation in an orbital shaker. The orbital shaker provides mixing during the activation process.

Anaerobic Activation

The anaerobic activation procedure involves the mixing of 10.0g of Biowish with 2.0L of tap water with an incubation time of 96 hours at 30°C. The mixture was placed in a 2L media bottle and sealed in preparation for agitation in an orbital shaker. The orbital shaker provides mixing during the activation process.

Plate Count

Following the activation procedure a plate count was performed to determine the number of viable colonies on a volumetric basis. The media from the activation procedure was allowed to settle for 30 minutes then 10mL of solution was extracted from the surface of the settled mixture. The solution was then diluted with factors of 10^{-5}, 10^{-6}, and 10^{-7} using a sterilized phosphate buffered saline solution and serial dilutions. Following dilution, 1mL of each solution was plated using plate count agar in pour plates. Each pour plate was allowed to incubate at 30°C for 48 hours then counted.

Denitrification

The denitrification experiment utilized sealed bioreactors of M9 minimal media (Crawford) with 200 mg/L-C glucose and 200mg/L-N nitrate as the carbon source and nitrogen source respectively. Three bioreactors of 2L M9 minimal media were then inoculated with Biowish activated under the standard procedure, anaerobic-nitrate procedure, and the anaerobic procedure. The inoculant consisted of 200mL of activated Biowish according to the standard procedure, anaerobic-nitrate procedure, and the anaerobic procedure. The activated Biowish was allowed to settle for 30 minutes and then the 200mL solution was extracted from the surface of the mixture. The inoculant was then mixed with the media and separated into five discrete 250mL sealable bioreactors to ensure the sampling procedure would not reoxygenate the remaining bioreactors. The 15 bioreactors where then allowed to react in an orbital mixer at 30°C for the appropriate time as outlined by the sampling procedure.

Samples were taken from each method of activation at 0,1,3,6, and 12 hours. The samples were immediately acidified to a pH less than 2 and filtered at 0.22 microns shortly after. The acidification serves to stop the denitrification reaction while the filtration serves to remove the bacteria from the media for total soluble nitrogen analysis.

The samples were then analyzed for nonpurgeable organic carbon and total nitrogen using a total organic carbon analyzer (Williams). The nonpurgeable organic carbon data indicates the remaining soluble glucose while the total nitrogen data indicates the remaining aqueous nitrogen.

Results

	10^-5 mL	10^-6mL	10^-7mL
Anaerobic-Nitrate Activation	Uncountable High	Uncountable High	242 CFU
Anaerobic Activation	Uncountable High	Uncountable High	72 CFU
Aerobic Activation	Uncountable High	Uncountable High	231 CFU

Figure 1: Plate Count Data

Figure 1 provides the number of colony forming units for the various activation procedures. It was prudent to show that each activation procedure allows for a similar quantity of bacteria in the inoculum as the bacteria facilitate the denitrification pathways.

Anaerobic-Nitrate Activation Denitrification	NPOC(mg/L-C)	TN(mg/L-N)
0 Hours	199.3	232.0
1 Hour	197.7	222.9
3 Hours	198.6	222.6
6 Hours	156.3	214.6
12 Hours	57.66	182.5

Figure 2: Anaerobic-Nitrate Activation Denitrification

Standard Activation Denitrification	NPOC(mg/L-C)	TN(mg/L-N)
0 Hours	217.2	199.6
1 Hour	217.0	199.5
3 Hours	214.2	198.5
6 Hours	208.3	195.6
12 Hours	80.57	161.0

Figure 3: Standard Activation Denitrification

Anaerobic Activation Denitrification	NPOC(mg/L-C)	TN(mg/L-N)
0 Hours	210.7	200.9
1 Hour	210.4	183.2
3 Hours	209.0	170.2
6 Hours	204.2	170.4
12 Hours	185.6	165.4

Figure 4: Anaerobic Activation Denitrification

After 12 hours the Anaerobic-Nitrate Activation Denitrification bioreactor was able to remove 49.5mg/L of nitrogen while the Standard Activation Denitrification bioreactor was able to remove 38.6mg/L of nitrogen, a 28 percent improvement. The Anaerobic Activation Denitrification achieved similar results to the Standard Activation denitrification. In all cases the majority of the denitrification happened between 6 to 12 hours.

The nitrogen to carbon usage rate in the Anaerobic-Nitrate Activation Denitrification bioreactor was 0.35. While the carbon usage rate in the Standard Activation Denitrification bioreactor was 0.28.

Discussion

The plate count was performed to ensure the Anaerobic-Nitrate Activation procedure will yield viable colony forming units in the same order of magnitude as the Aerobic Activation procedure. As the denitrifying bacteria in Bio wish are facultative, the plate count will contain both denitrifiers and nondentirfying bacteria.

The Anaerobic-Nitrate Activation Denitrification bioreactor was able to outperform both the Standard Activation Denitrification bioreactor and the Anaerobic Activation Denitrification bioreactor. Therefore confirming an anaerobic-nitrate activation procedure for Biowish will increase denitrification rates when compared to the standard aerobic activation procedure.

The denitrification from 6 to 12 hours in the cases of both Anaerobic-Nitrate and Aerobic activation removed 32 and 34 mg/L of nitrogen respectively. As indicated by previous Biowish denitrification experiments (Lee) Biowish denitrification follows zero order decay; furthermore, after inoculation a lag occurs before denitrification can be exhibited. However, after activation in anaerobic-nitrate conditions Biowish was able to denitrify without delay resulting a 28 percent increase in nitrogen removal after 12 hours.

Further experimentation can be performed to determine if the increased denitrification rate will be sustained in a sequential batch reactor configuration. The bacterial concentration should converge to a similar concentration given enough sequences therefore nullifying the denitrification gains from anaerobic-nitrate activation. Furthermore, any prepackaged multi-bacterial product could have significant process improvements if the culture is incubated under conditions similar to the conditions found in the intended application.

Biowish will be a more effective denitrification inoculum if the anaerobic-nitrate activation protocol outlined in the methods section is used over the standard activation protocol.

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